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Supplemental Material to:

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ESX-1 dependent impairment of autophagic flux by *mycobacterium tuberculosis* in human dendritic cells

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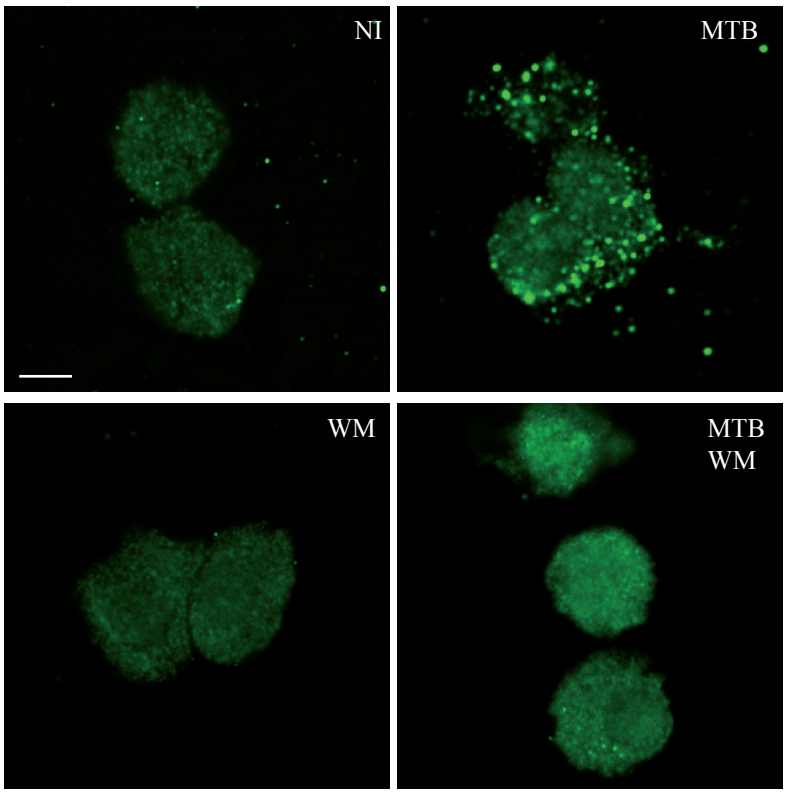
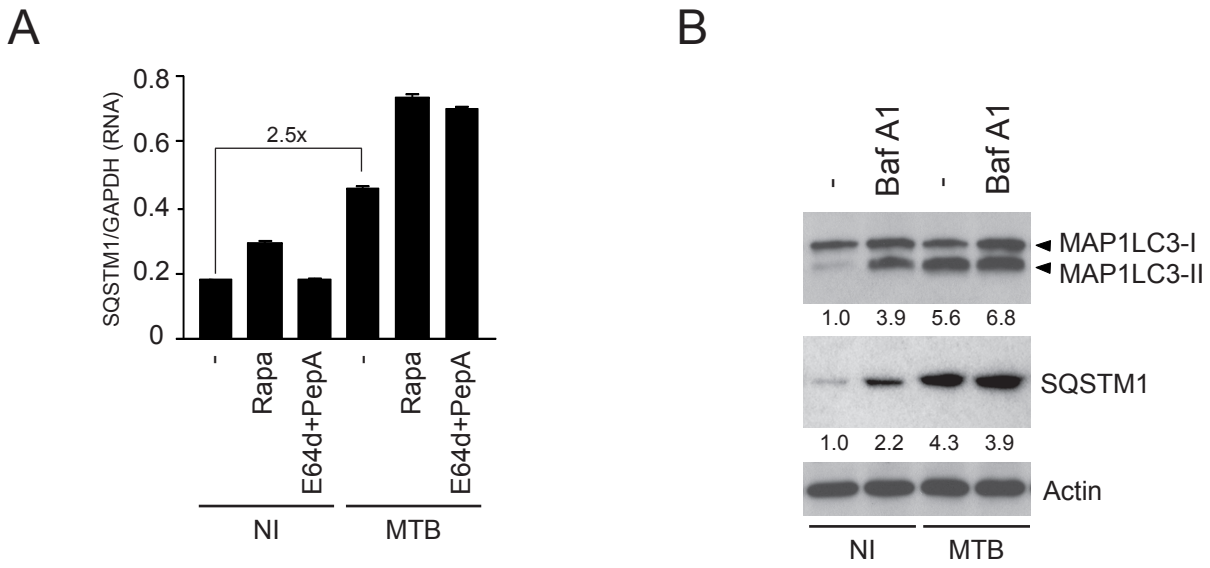
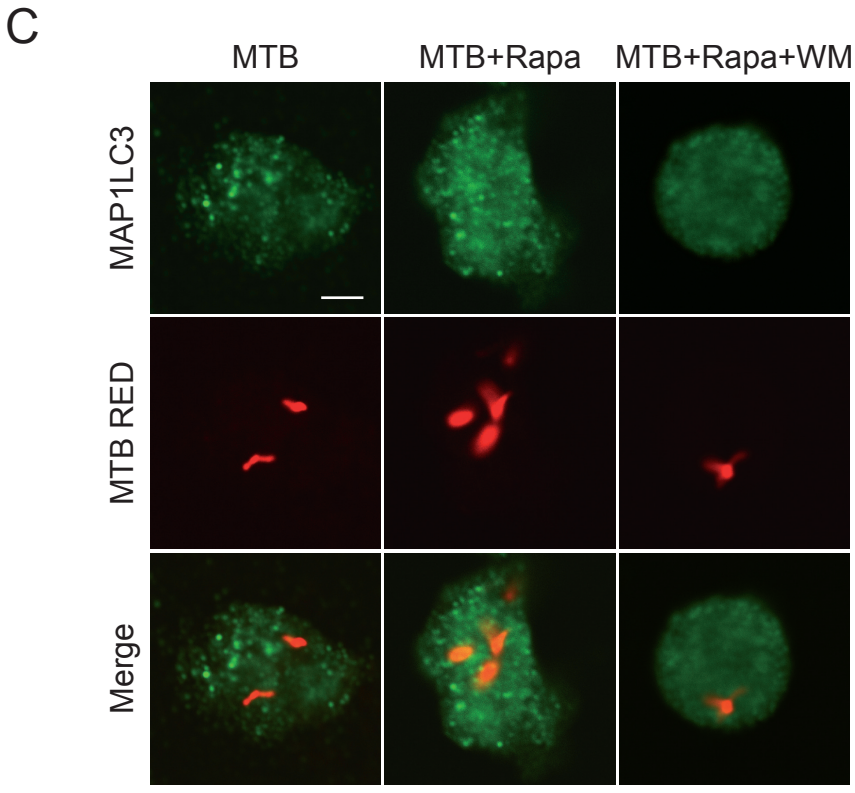
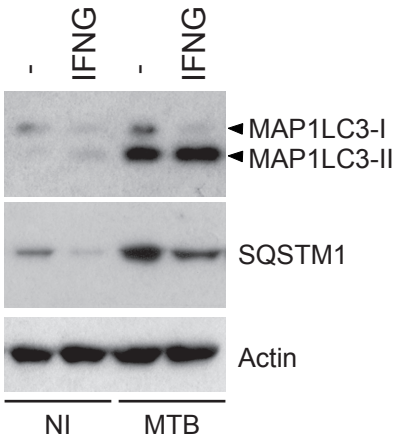
Figure S1



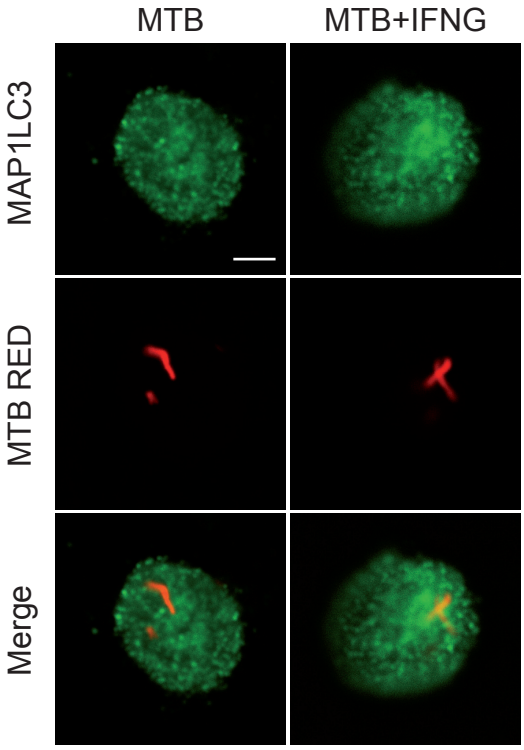

A
SQSTM1/GAPDH (RNA)
0.8
0.6
0.4
0.2
0
2.5x
-
Rapa
E64d+PepA
-
Rapa
E64d+PepA
NI
MTB
B
-
Baf A1
-
Baf A1
MAP1LC3-I
MAP1LC3-II
1.0
3.9
5.6
6.8
SQSTM1
1.0
2.2
4.3
3.9
Actin
NI
MTB


C
MTB
MTB+Rapa
MTB+Rapa+WM
MAP1LC3
MTB RED
Merge

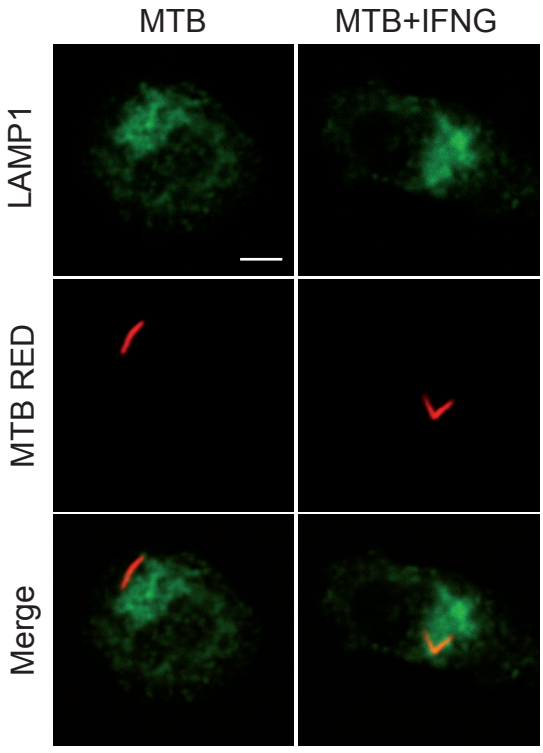
A


-
IFNG
-
IFNG
MAP1LC3-I
MAP1LC3-II
SQSTM1
Actin
NI
MTB

B


MTB
MTB+IFNG
MAP1LC3
MTB RED
Merge

C


MTB
MTB+IFNG
LAMP1
MTB RED
Merge

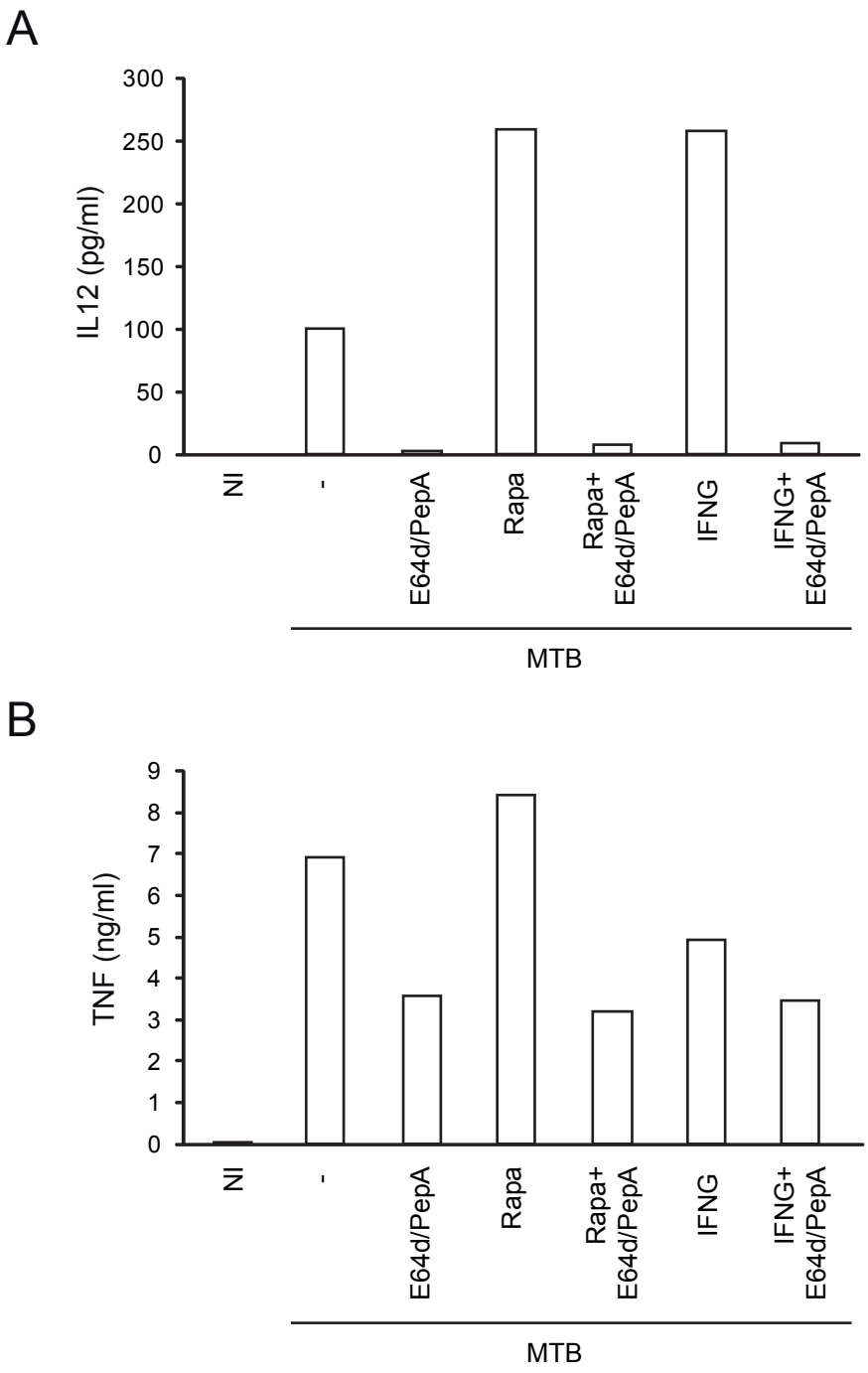

A
IL12 (pg/ml)
300
250
200
150
100
50
0
NI
-
E64d/PepA
Rapa
Rapa+ E64d/PepA
IFNG
IFNG+ E64d/PepA
MTB
B
TNF (ng/ml)
9
8
7
6
5
4
3
2
1
0
NI
-
E64d/PepA
Rapa
Rapa+ E64d/PepA
IFNG
IFNG+ E64d/PepA
MTB

Figure S5

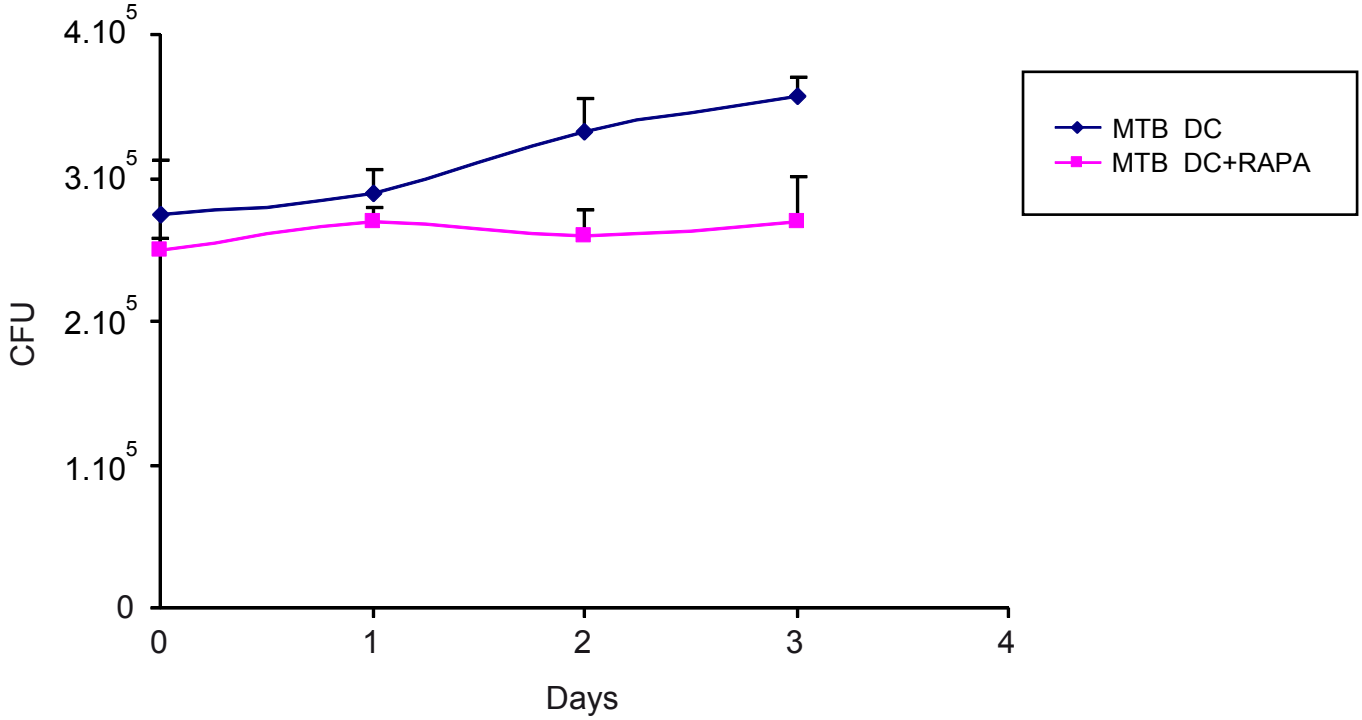


Figure S1. Effects of wortmannin on Mtb-induced MAP1LC3 accumulation. DC were infected with Mtb and treated with wortmannin (WM 0.2 μM; 2 h after infection). After 24 h, autophagy levels were analysed for MAP1LC3 expression by immunofluorescence using an MAP1LC3 antibody. Scale bar, 6 μm. NI: not infected DC.

Figure S2. Analysis of autophagic flux in Mtb-infected DC. (A) Real-time RT-PCR for SQSTM1 and GAPDH was performed on RNA samples obtained from DC stimulated as described in Fig. 2A. Levels of SQSTM1 mRNA are normalized to the GAPDH level using the formula $2^{-\Delta Ct}$; the values shown are means ± SD of triplicate determinations. (B) DC were infected with Mtb and treated with Bafilomycin A1 (Baf A1 25 μM, 4 h before cell harvesting) or left untreated (vehicle, DMSO). Cells were harvested 24 h after infection and analysed for MAP1LC3 and SQSTM1 levels by immunoblotting. The results shown are from one of three experiments that yielded similar results. NI: not infected DC. Actin levels were analysed to verify protein amount loading. Quantification of the MAP1LC3 and SQSTM1 levels were indicated at the bottom of of each immunoblot. (C) Mtb RED-infected cells were incubated with Rapamycin, as described in Fig. 2A, and/or Wortmannin (WM 0.2 μM; 3h post infection), fixed 24 h after infection and stained for MAP1LC3. The images displaying the merge of the two fluorescence signals (Red: Mtb; Green: MAP1LC3) are shown on the bottom panels. Scale bar, 6 μm.

Figure S3. Effects of IFNG on the inhibition of autophagic flux caused by Mtb infection. (A) DC were infected with Mtb and treated with IFNG (10 ng/ml, 4 h after infection) or left untreated. Cells were harvested 24 h after infection and analysed for MAP1LC3 and

SQSTM1 levels by immunoblotting. The results shown are from one of three experiments that yielded similar results. NI: not infected DC. Actin levels were analysed to verify protein amount loading. (B, C) Mtb RED-infected cells were incubated with IFNG as described in (A), fixed 24 h after infection and stained for MAP1LC3 (Red: Mtb; Green: MAP1LC3) (B) or for LAMP1 (Red: Mtb; Green: LAMP1) (C). The images displaying the merge of the two fluorescence signals are shown on the bottom panels. Scale bar 6 μm.

Figure S4. Effect of E64D/PepA treatment on IL12 and TNF secretion from Mtb-infected DC in presence of autophagy inducers. Cell culture supernatants were collected from DC cultures infected with Mtb and treated with Rapamycin, as in Fig. 2A, or IFNG as described in Fig. S3A and/or E64d plus PepA A (E64d+PepA 10 μg/ml each, 4 h after infection). The production of IL12 (A) and TNF (B) was measured by CBA Flex Set. NI: not infected DC.

Figure S5. Effect of Rapamycin on Mtb viability on infected DC. DC were infected with Mtb and treated with Rapamycin (Rapa 0.2 μM; 4 h after infection). At the indicated time points, cells were lysed to determine the number of viable bacteria by plating CFU. Values are expressed as a mean of three independent experiments ± SD.